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# Isolation, cultural and physiological characterisation of *Azospirillum* from acidic soils of Ranchi

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**Abstract**
*Azospirillum* spp. is of worldwide distribution prevalent in tropical, sub-tropical and temperate climatic conditions and has been isolated from the rhizosphere of a variety of tropical and sub-tropical non-leguminous crops. Keeping these points in view a study was conducted during *Rabi* and *Kharif* 2016-17 in the Department of Soil Science and Agricultural Chemistry, Birsa Agricultural University, Ranchi, Jharkhand to screen out the presence of *Azospirillum* in rhizosphere of various non-leguminous crops and to characterize the isolates on the basis of cultural and physiological behaviours. On the basis of pH range (4.0-5.5), 54 soil samples were tentatively selected out of 100 samples for investigation. From the study conducted, presence of *Azospirillum* in rhizosphere of lower pH was confirmed. 6 colonies out of 54 colonies were translucent while the rest were opaque in terms of degree of opacity. Amount of growth ranged from large to slight. Growth pattern of colonies were found filiform and beaded in 41 and 13 colonies respectively. Physiological investigation revealed that *Azospirillum* isolates were microaerophilic in relation to free oxygen i.e., developed white pellicles 1-2 mm below the surface of semi-solid Okon's media after 48 hours of incubation at 35°C. All isolates were positive for catalase reaction but responded negative for Indole production and Gelatin liquification test. 15 isolates out of 54 were able to hydrolyse starch. 20 isolates responded positive to nitrate reduction test. 19 isolates out of 54 required biotin for their growth. Pigmentation was reported in 13 isolates after 48 hours of incubation at 35°C on Okon's solid agar media.

**Keywords:** *Azospirillum*, rhizosphere, microaerophilic, gelatin, indole

## Introduction
The concept of Rhizosphere was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities (Hiltner, 1904) [8]. Rhizosphere is a potent environment which harbours diverse group of microbial activity and from this zone, some bacteria which are beneficial for plants are termed as 'rhizobacteria'. There are two types of rhizobacteria on the basis of their association with plants, one that can form a symbiotic relationship with the plant, and other that are free-living and interact with the roots in the soil (Glick, 2005) [7]. Root exudates are the substrate or fuel for the intense microbial (bacteria, fungi, algae, protozoa, nematodes and arthropods) activity within the rhizosphere (Lugtenberg *et al.* 2002) [13]. *Azospirillum* spp. have been identified mainly as rhizosphere bacteria and its colonization of the rhizosphere has been studied extensively in various non-leguminous crops (Steenhoudt and Vanderleyden 2000) [24]. The soil bacterium *Azospirillum* was first isolated from the Netherlands and originally named as *Spirillum lipoferum* by Beijerinck *et al.* (1925) [4]. Later Schroder (1932) [21] isolated from the soils in Germany and Austria. Till now, they have been isolated from the rhizosphere of many grasses and cereals all over the world, in a wide variety of terrestrial and aquatic habitats of tropical as well as in temperate climates (Yooshinan, 2001) [28]. Its occurrence in the rhizosphere varied from 1 to 10 per cent to the total rhizosphere population (Okon, 1985) [16]. Occurrence of *Azospirillum* in soil is strongly pH-dependent with a pH around 7, being optimal. However, sporadic occurrence was observed even in soils with pH 4.8 (Magalhaes *et al.* 1983) [14]. Fundamental idea about the physiological and cultural behaviours of PGPRs is pivotal for understanding diverse aspects related to rhizosphere performance and successful interactions with plant roots. This knowledge might give perception about the type of colonization of root surfaces by PGPRs, the way through which these microorganisms benefit plants and might stimulate ideas about how to improve the production and application of PGPR inoculants. Hence the present work was undertaken with a view to screen out the presence of *Azospirillum* spp. from the rhizosphere of acidic soils of Ranchi (Jharkhand) and characterise them on the basis of their cultural and physiological behaviour.

## Materials and Methods

### Material

*Azospirillum* species studied in the present investigation were isolated from soil of rhizosphere having pH range of 4.0 to 5.5 of different non-leguminous crops grown in various blocks *viz.*, Kanke, Aangara, Nagri, Bero, Itki of Ranchi district. Details of the location, soil pH and crop grown selected for isolation of *Azospirillum* are mentioned in Table 1.

### Collection of rhizosphere soil

Rhizosphere soils were collected from the rhizospheric region of the plant at the depth of 5-6 cm near the periphery of roots of different crops from different blocks of Ranchi district in plastic bags. The soil samples were preserved in refrigerator.

### pH of soil samples

Soil samples were collected from 100 different locations from Ranchi districts for pH analysis. The soil samples were air dried, grinded and sieved for estimation of pH by adopting standard methods**.** Soil pH was determined in a soil water suspension of 1:2.5 w/v, stirred at regular intervals for 30 minutes using pH meter (Jackson 1973) [11].

**Table 1:** Details of selected 54 soil samples selected for isolation of *Azospirillum*

| S. No. | Sample. No. | Place of collection | pH of the soil | Crop (previous/ present) |
|---|---|---|---|---|
| 1 | AZM5 | B.A.U Campus, SSAC, Kanke block | 5.4 | Maize |
| 2 | AZM6 | B.A.U Campus, SSAC, Kanke block | 5.3 | Maize |
| 3 | AZM10 | B.A.U Campus, SSAC, Kanke block | 5.5 | Rice |
| 4 | AZM15 | B.A.U Campus, Tech park, Kanke block | 5.1 | Rice |
| 5 | AZM16 | B.A.U Campus, Tech park, Kanke block | 5.4 | Ragi |
| 6 | AZM17 | R.A.C Farm, W-section, Kanke block | 5.3 | Rice |
| 7 | AZM18 | R.A.C Farm, W-section, Kanke block | 5.1 | Ragi |
| 8 | AZM19 | R.A.C Farm, W-section, Kanke block | 5.2 | Rice |
| 9. | AZM22 | R.A.C Farm, W-section, Kanke block | 5.5 | Wheat |
| 10. | AZM23 | R.A.C Farm, W-section, Kanke block | 5.4 | Wheat |
| 11. | AZM25 | Chamghati, Aangara block | 5.5 | Rice |
| 12. | AZM26 | Chamghati, Aangara block | 5.3 | Rice |
| 13. | AZM27 | Chamghati, Aangara block | 5.1 | Rice |
| 14. | AZM29 | Chamghati, Aangara block | 5.2 | Rice |
| 15. | AZM30 | Chamghati, Aangara block | 5.4 | Rice |
| 16. | AZM32 | Chamghati, Aangara block | 5.4 | Rice |
| 17. | AZM33 | Chamghati, Aangara block | 5.3 | Rice |
| 18. | AZM34 | Chamghati, Aangara block | 5.2 | Rice |
| 19. | AZM35 | Chauli patra, Nagri block | 4.9 | Pea |
| 20. | AZM36 | Chauli patra, Nagri block | 4.6 | Ragi |
| 21. | AZM 39 | Itki mor, Itki block | 4.7 | Potato |
| 22. | AZM 40 | Itki mor, Itki block | 4.6 | Ragi |
| 23. | AZM 42 | Itki mor, Itki block | 4.7 | Mustard + Pea |
| 24. | AZM 45 | Garhgao, Itki block | 4.6 | Pea + Sugarcane |
| 25. | AZM 46 | Garhgao, Itki block | 5.1 | Wheat |
| 26. | AZM 53 | Devali, Itki block | 5.4 | Ragi |
| 27. | AZM 55 | Devali, Itki block | 4.7 | Potato |
| 28. | AZM 56 | Devali, Itki block | 4.8 | Maize |
| 29. | AZM 60 | Bhandra, Itki block | 4.2 | Maize |
| 30. | AZM 61 | Bhandra, Itki block | 4.7 | Onion |
| 31 | AZM 62 | Karmatoli, Bero block | 4.1 | Pea + Potato |
| 32. | AZM 63 | Karmatoli, Bero block | 4.0 | Potato |
| 33. | AZM 64 | Karmatoli, Bero block | 4.0 | Potato |
| 34. | AZM 65 | Kalanji, Bero block | 4.0 | Ginger |
| 35. | AZM 66 | Didhiya, Bero block | 4.1 | Mustard + Pea |
| 36. | AZM 70 | Tuko, Bero block | 5.1 | Pea |
| 37. | AZM 71 | Tuko, Bero block | 4.4 | Potato |
| 38. | AZM 75 | Parepara, Bero block | 4.6 | Pea |
| 39. | AZM 76 | Parepara, Bero block | 4.9 | Potato |
| 40. | AZM 77 | Parepara, Bero block | 4.7 | Lentil |
| 41. | AZM 80 | Jainathpur, Bero block | 4.8 | Pea |
| 42. | AZM 81 | Jainathpur, Bero block | 4.9 | Mustard |
| 43. | AZM 83 | Bhaishmuro, Bero block | 4.4 | Ginger |
| 44. | AZM 84 | Bhaishmuro, Bero block | 4.8 | Mustard |
| 45. | AZM 85 | Bhaishmuro, Bero block | 4.4 | Pea |
| 46. | AZM 87 | Bhaishmuro, Bero block | 4.1 | Ragi |
| 47. | AZM 88 | Bhaishmuro, Bero block | 4.3 | Potato |
| 48. | AZM 89 | Bhaishmuro, Bero block | 4.4 | Potato |
| 49. | AZM 90 | Bhaishmuro, Bero block | 4.1 | Potato |
| 50. | AZM 93 | Kundo, Bero block | 4.2 | Potato |
| 51. | AZM 94 | Kundo, Bero block | 4.8 | Ragi |
| 52. | AZM 95 | Kundo, Bero block | 4.6 | Maize |
| 53. | AZM 99 | Bero, Bero block | 4.3 | Potato |
| 54. | AZM 100 | Bero, Bero block | 4.7 | Ragi |

**Isolation and purification of *Azospirillum* spp.**

Isolation of *Azospirillum* species from rhizospheric soils was done following the methods of serial dilution. From the soil samples selected on the basis of pH range (4.0-5.5), 1 g of soil was taken and serially diluted using sterile distilled water upto $10^{-6}$ dilutions. One ml of diluted sample from $10^{-4}$ to $10^{-6}$ dilutions were taken and 1ml of aliquot was inoculated in tubes containing Okon's Nfb (Nitrogen free bromothymol) semi-solid media. All the tubes were incubated at 35°C for 48 h and observed the growth by the formation of pellicles. Pellicles formation is considered as positive for *Azospirillum.* Pellicles were streaked on petriplates containing Nfb Okon's solid media and incubated at 35°C for 48 hours. Morphologically divergent *Azospirillum* colonies (white, yellow and pink) were picked from the plates of dilution $10^{-5}$ and streaked on basal minimal salt agar medium and incubated at 35°C for 24 h. After attaining sufficient growth, all the isolates were preserved in a refrigerator for further investigation. The colonies developed on Okon's agar medium (pH adjusted to 6.8) were transferred to slants of same medium and stored at $4^0$C.

**Okon's Media**

Malic acid 5.00 g, KOH 4.00 g, $K_2HPO_4$ 0.50 g, $FeSO_4.7H_2O$ 0.05 g, $MnSO_4.7H_2O$ 0.01g, $MgSO_4.7H_20$ 0.10g, NaCl 0.02 g, $CaCl_2$ 0.01g, $Na_2MoO_4$ 0.002g, Bromothymol blue (0.5% in 95% methanol) 2.00 ml, Agar 1.8 g (semi-solid)/18 g(solid), $NH_4Cl$ 1 g and Water 1 ltr.

**Purification of the culture**

Purification of the culture was carried out by frequent transfer of colony of *Azospirillum* developed on Okon's agar media to seal solid nitrogen free malate medium on petriplates (Okon *et al*. 1977) [17] having the following constituents: $K_2HPO_4$ 6.0 g, $KH_2PO_4$ 4.0 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, $CaCl_2$ 0.2 g, $NH_4Cl$ 0.1 g, NaOH 3.0 g, Yeast extract 0.1 g, $FeCl_3$ 10.0 mg, $Na_2MoO_4$ 20.00 mg, $MnSO_4$ 2.10 mg, $H_3BO_3$ 2.80 mg, $Cu(NO_3)_2$ 0.04 mg, Agar 18 g, Water 1 ltr

**Cultural characterization**

Different isolates of *Azospirillum* species were grown on respective standard media and their characteristic growth patterns were observed. Serially diluted isolates of *Azospirillum* species were grown on Okon's agar medium (Okon *et al*. 1977) [17] in petriplates and in tubes (for agar strokes) at $35^0$C for 72 hours then purification of colonies were done. Observations were made with regard to nature of colonial growth.

**Physiological characterization**

The following tests were performed to study the physiological behavior of the isolates. In general standard techniques as described in the Manual of Microbiological Methods (1957) and Bergey's manuals of determinative Bacteriology 6th Edition were followed. However in certain test specific method (mentioned against the tests) are also carried out.

**Relation to free oxygen**

*Azospirillum* cultures were inoculated in solid malate medium contained in screw capped vials and mixed thoroughly by rotating the tubes and incubated at for a week. Duplicate tubes were put for each isolate aforesaid observations were recorded regarding position of growth, thereby, indicating the oxygen requirement. Following observations were recorded regarding position of growth in the culture tube

Growth on the surface of the medium: Aerobic
Growth just below the upper surface of medium: Microaerophilic
Growth deep in the tube: Anaerobic

**Catalase reaction**

A loopful of 48 hours grown cultures from respective media semi solid malate medium for *Azospirillum* was taken on a glass slide and one ml of 10 percent $H_2O_2$ was poured over it. Production of gas bubbles from the cultures was taken as positive for catalase (Aneja 1993) [1].

**Production of pigment**

In case of *Azospirillum* slants containing Okon's agar medium were inoculated with loopful of 48 hours old cultures and incubated at $35^0$C. Pigmentation produced in duplicate tubes were recorded two weeks after incubation.

**Gelatin Liquefaction**

Plates containing nutrient agar with 0.4 percent gelatin (sterilized at 115 $^0$C for 20 minutes) were streaked with respective test isolates and incubated for 15 days at their respective temperature optima. Duplicate plates were kept for each isolate. Observation were recorded for the appearance of yellow halo around the growth indicates the utilization of gelatin (Stolpe and Godkeri 1981) [25] after flooding colonies with mercuric chloride solution ($HgCl_2$ 15 g, conc. HCl 20ml, distilled water 100 ml).

**Hydrolysis of Starch**

Plates containing nutrient agar and 1.0 percent soluble starch (Steam sterilized) were streaked with respective isolates in centre and incubated at $35^0$C (*Azosprillum*) for two weeks. Duplicate plates were kept for each isolate along with inoculated control. Observations for change in colour around streaks after flooding with dilute iodine solution reflected the extent of hydrolysis of starch as against control. A colourless halo around the growth and blue colour in the rest of the plates showed utilization of starch by the microorganisms (Stolpe and Godkeri 1981) [25].

**Production of Indole**

Ten milliliter tryptone broth (Tryptone 1%, NaCl 0.15%, pH 7.2) was dispensed in test tubes and steam sterilized at $121^0$C for 20 minutes. Tubes were inoculated maintained for each isolate alongwith the control and incubated at $35^0$C for two weeks for *Azospirillum* respectively. Production of indole was treated by adding 0.5ml amyl alcohol, 75ml conc HCL 25ml) to each tube. Formation of deep red colour in alcohol layer are taken as criterion for Indole production (Seeley and Vandemark 1981) [22].

**Nitrate reduction**

In case of *Azospirillum* isolates, the method of Neyra *et al*. (1977) [15] was adopted with the following composition of semisolid medium as: Malic acid 5.00g, KOH 4.00g, $K_2HPO_4$ 0.50g, $MgSO_4.7H_2O$ 0.20g, NaCl 0.10g, $CaCl_2$ 0.02g, $FeSO_4.7H_2O$ 0.50g, $MnSO_4$, $H_2O$ 0.10g, $Na_2MoO_4.2H_2O$ 0.002g, Bromothymol blue (0.5% in 95% alcohol) 2.0ml, Agar 1.75g, $NH_4NO_3$ 10.00mM, Distilled water 1 litre. Five milliliter of the above medium was dispensed in tubes and sterilized. Inoculation was done with 0.5 ml of culture suspension. Duplicate tubes for each isolate including control were incubated at $35^0$C for 7 days.

Reduction of nitrate to nitrite was tested by adding 1 ml each of the reagents ; a) Sulphanilic acid 8 g in 1000ml of 5N acetic acid, b) Naphthylamine 5 g in 1000 ml of 5N acetic acid. Production of pink colour indicated the presence of nitrite (reduction of nitrate). Reduction of nitrate was tested on 7th day of incubation.

**Biotin requirement of *Azospirillum* isolates**

Medium proposed by Tarrand *et al.* (1978) [26] consisting of $K_2HPO_4$ 0.5g, Succinic acid 5.0g, $FeSO_4.7H_2O$ 0.01g, $Na_2MoO_4.2H_2O$ 0.002g, $MgSO_4$ 0.2g, NaCl 0.1g, $CaCl_2.2H_2O$ 0.026g, $(NH_4)_2SO_4$ 1.0g (pH 7.0 with KOH) with and without (0.0001 g per liter) were prepared. Five milliliter incubated at $37^0$C for 48 hours after inoculation with different isolates.

Culture grown in Okon's broth were inoculated in 25 ml of nutrient broth and inoculated for $35^0$C for 48 hours. The cells were harvested by centrifugation and washing twice in 100 ml of sterilized water. Finally, a cell suspension having turbidity of 20 Klett unit (*blue filter*) was prepared and 0.1 ml of this suspension was used to inoculate each culture tube prepared as mentioned above and incubated for 48 hours at $35^0$C. Visual observation regarding extent of growth of cells in tubes containing biotin was compared with the growth without biotin.

**Results and discussions**

In the present study, selectivity to grow on specific Nfb (Nitrogen free bromothymol) media and subsequently confirming their morphological, cultural and physiological identity with the type cultures as described in Bergey's Manual (Buchanan and Gibbons, 1974) [5] and *Aquaspirillum* taxonomy for *Spirillum* (Kreig and Hylemon, 1976) [12] were taken as reference for investigation and characterization of *Azospirillum* isolates. A total of 54 isolates were studied under various cultural and physiological behaviours which are presented in Table 2.

**Cultural Characteristics**

**Colony morphology**

Study revealed that colonies developed on agar slants were smooth, 6 out of 54 colonies were translucent while rest were opaque in terms of degree of opacity. Amount of growth ranged from large to slight. Growth pattern of colonies were filiform and beaded in 41 and 13 colonies respectively. This might be inferred from the investigation that *Azospirillum* displays high degree of pleomorphism with cellular and colony variations among the species as well as within each species depending on the strain, medium composition and culture conditions as reported by Becking, 1985 [3]. The same was investigated by Rasool *et al.* (2015) [15].

**Physiological characteristics**

**Relation to free Oxygen**

Oxygen being an ultimate electron acceptor in the electron carrier chain the organism, catabolizing the carbon substrate through aerobic respiratory pathway is required in optimal concentration in the cell. Thus each organism according to their need establishes a physiological relationship with oxygen. Such relationship has been demonstrated in the present study in the case of *Azospirillum* grown in nitrogen-free shake culture.

All 54 isolates of *Azospirillum* grown in nitrogen-free semi-solid malate medium exhibited their growth in the form of a pellicle just below the upper surface (1-2 mm below the surface) of the medium indicating thereby the requirement of low partial pressure of Oxygen under dinitrogen fixing condition. Hence they are microaerophilic in relation to free oxygen. This finding is confirmatory with the investigations carried out by Reis *et al.* (2006) [19].

**Catalase reaction**

Physiological relationship of bacteria to oxygen is determined by the number and variety of enzymes capable of reacting with oxygen. The oxidation of flavo-proteins by oxygen invariably result in the formation of a toxic compound, Hydrogen peroxide ($H_2O_2$) and also to a lesser extent even more toxic free radical, Superoxide ($O_2^-$). Most of the aerobes and facultative anaerobes have been reported to contain enzymes like superoxide dismutase, catalase and peroxidase while aero tolerant anaerobes contain only superoxide dismutase for their roles in protecting the cell from toxic consequences of oxygen metabolism (Stanier *et al.* 1977) [23]. Investigation revealed that all 54 isolates of *Azospirillum* were positive for catalase reaction. It is due to presence of enzyme catalase in all the strains of *Azospirillum*. The findings are in conformity with the works of Ilyas *et al.* (2012) [10].

**Indole production test**

All the isolates were negative for Indole production. *Azospirillum* is not capable to synthesize indole from tryptophan. This result was in conformity with the findings of Rosemary *et al.* (2013) [20].

**Starch hydrolysis test**

Out of 54 isolates, 15 isolates were able to hydrolyse starch due to presence of enzyme amylase while rest could not hydrolysed starch. This result was in conformity with the findings of Usha and Kanimozhi (2011) [27] and Hossain *et al.* (2015) [9].

**Nitrate reduction test**

Investigation revealed that 20 isolates out of 54 were capable for nitrate reduction in case of shortage of oxygen for their metabolic process. The results were in confirmity with the work of Baliah and Rajalaxmi (2015) [2].

**Production of pigments**

By investigation it was obsevered that pigmentation was found in 13 isolates out of 54 while rest isolates did not developed any pigmentation. This was due to presence of carotenoid pigments in that specific isolates. The same was reported by Rasool *et al.* (2015) [18].

**Biotin requirement**

The *Azospirillum* isolates responded differentially to biotin requirement. 19 isolates out of 54 require biotin for their growth. Rest did not required biotin for their growth. The same was also reported by Baliah and Rajalaxmi (2015) [2].

**Gelatin Liquification**

Results revealed that all 54 isolates of *Azospirillum* responded negative to the gelatin liquification test. Gelatin hydrolysis test is used to detect the ability of an organism to produce gelatinase (proteolytic enzyme) that liquefy gelatin. *Azospirillum* strain is not capable to liquify gelatin due to absence of enzyme gelatinase. This result is in conformity with the findings of Hossain *et al.* (2015) [9].

## Conclusion

Free living diazotroph *Azospirillum* are able to survive even at pH 4.0 i.e., under highly acidic conditions and they have wider availability in rhizospheric soils of different blocks of Ranchi district. *Azospirillum* spp. show high degree of polymorphism in respect to their colonial patterns, opacity etc which may be attributed to their isolation from different rhizospheric and soil conditions where they were surviving. Cultural characterization revealed that colonies of *Azospirillum* developed were translucent to opaque in terms of degree of opacity. Growth patterns varied from slight to large. Shape of the colonies was either filliform or beaded. Physiological characterizations revealed that *Azospirillum* isolates grow under Microaerophillic condition i.e., developed as sub-surface pellicle (1-2 mm below the surface) in Nitrogen free semi solid malate media. All isolates were positive for catalase test but negative for indole production test. *Azospirilla* isolates were unable to liquify gelatin. They grew better at 35°C and the optimum pH for growth was 6.8. Pigmentation were observed in some of the isolates while other isolates did not developed any pigmentation. This is due to the presence of carotenoid pigments. Few isolates were able to hydrolyse starch due to the presence of enzyme amylase for meeting their carbon requirements. Some isolates required Biotin for their growth while rest were able to grow without Biotin. Moreover few isolates were also able to reduce nitrate ($NO_3^-$) into nitrite ($NO_2^-$).

**Table 2:** Physiological and cultural characterization of new selected *Azospirillum* isolates

| Sl. No. | Isolates | Catalase reaction | Pigment | Starch hydrolysis | Nitrate reduction | Relation to free $O_2$ | Indole production | Biotin requirement | Gelatin liquification | Growth | Opacity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | AZM 5 | Present | - | - | - | Microaerophillic | - | Not required | - | Large, Filiform | Opaque |
| 2. | AZM 6 | Present | - | - | - | Microaerophillic | - | Not required | - | Large, Filiform | Opaque |
| 3. | AZM 10 | Present | - | - | - | Microaerophillic | - | Not required | - | Large, Filiform | Translucent |
| 4. | AZM 15 | Present | - | - | - | Microaerophillic | - | Not required | - | Large, Filiform | Translucent |
| 5. | AZM 16 | Present | - | - | - | Microaerophillic | - | Required | - | Large, Filiform | Translucent |
| 6. | AZM 17 | Present | - | - | - | Microaerophillic | - | Not required | - | Large, Filiform | Translucent |
| 7. | AZM 18 | Present | - | - | - | Microaerophillic | - | Required | - | Large, Filiform | Opaque |
| 8. | AZM 19 | Present | - | - | - | Microaerophillic | - | Required | - | Large, Filiform | Translucent |
| 9. | AZM 22 | Present | - | - | + | Microaerophillic | - | Required | - | Large, Beaded | Opaque |
| 10. | AZM 23 | Present | - | - | + | Microaerophillic | - | Required | - | Large, Beaded | Opaque |
| 11. | AZM 25 | Present | - | - | - | Microaerophillic | - | Required | - | Moderate, Filiform | Translucent |
| 12. | AZM 26 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Beaded | Opaque |
| 13. | AZM 27 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Beaded | Opaque |
| 14. | AZM 29 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Beaded | Opaque |
| 15. | AZM 30 | Present | - | - | - | Microaerophillic | - | Required | - | Moderate, Filiform | Opaque |
| 16. | AZM 32 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 17. | AZM 33 | Present | - | - | - | Microaerophillic | - | Required | - | Moderate, Filiform | Opaque |
| 18. | AZM 34 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 19. | AZM 35 | Present | - | - | + | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 20. | AZM 36 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 21. | AZM 39 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 22. | AZM 40 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 23. | AZM 42 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 24. | AZM 45 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 25. | AZM 46 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 26. | AZM 53 | Present | - | - | + | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 27. | AZM 55 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 28. | AZM 56 | Present | - | - | - | Microaerophillic | - | Required | - | Moderate, Filiform | Opaque |
| 29. | AZM 60 | Present | - | - | - | Microaerophillic | - | Required | - | Moderate, Filiform | Opaque |
| 30. | AZM 61 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 31. | AZM 62 | Present | + | + | + | Microaerophillic | - | Required | - | Slight, Beaded | Opaque |
| 32. | AZM 63 | Present | + | + | + | Microaerophillic | - | Required | - | Slight, Beaded | Opaque |
| 33. | AZM 64 | Present | + | + | + | Microaerophillic | - | Required | - | Slight, Beaded | Opaque |
| 34. | AZM 65 | Present | + | + | + | Microaerophillic | - | Required | - | Slight, Beaded | Opaque |
| 35. | AZM 66 | Present | + | + | + | Microaerophillic | - | Not required | - | Slight, Filiform | Opaque |
| 36. | AZM 70 | Present | + | + | + | Microaerophillic | - | Not required | - | Slight, Filiform | Opaque |
| 37. | AZM 71 | Present | + | + | + | Microaerophillic | - | Not required | - | Slight, Filiform | Opaque |
| 38. | AZM 75 | Present | - | - | + | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 39. | AZM 76 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 40. | AZM 77 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 41. | AZM 80 | Present | + | + | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 42. | AZM 81 | Present | + | + | + | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 43. | AZM 83 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 44. | AZM 84 | Present | - | - | + | Microaerophillic | - | Required | - | Moderate, Filiform | Opaque |
| 45. | AZM 85 | Present | - | - | + | Microaerophillic | - | Required | - | Moderate, Filiform | Opaque |
| 46. | AZM 87 | Present | + | + | + | Microaerophillic | - | Not required | - | Slight, Beaded | Opaque |
| 47. | AZM 88 | Present | - | + | + | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 48. | AZM 89 | Present | - | + | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 49. | AZM 90 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 50. | AZM 93 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 51. | AZM 94 | Present | - | - | - | Microaerophillic | - | Not required | - | Moderate, Filiform | Opaque |
| 52. | AZM 95 | Present | + | + | + | Microaerophillic | - | Required | - | Slight, Beaded | Opaque |
| 53. | AZM 99 | Present | + | + | + | Microaerophillic | - | Required | - | Slight, Beaded | Opaque |
| 54. | AZM 100 | Present | + | + | + | Microaerophillic | - | Required | - | Slight, Beaded | Opaque |

**Fig 1:** White translucent and beaded colonies of *Azospirillum*

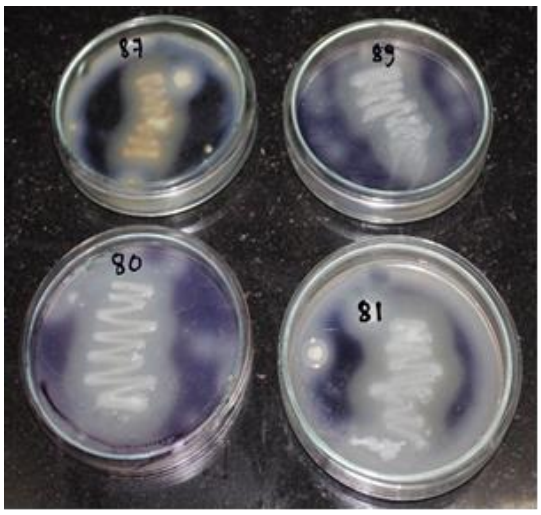


**Fig 2:** Isolates showing positive response to starch hydrolysis test

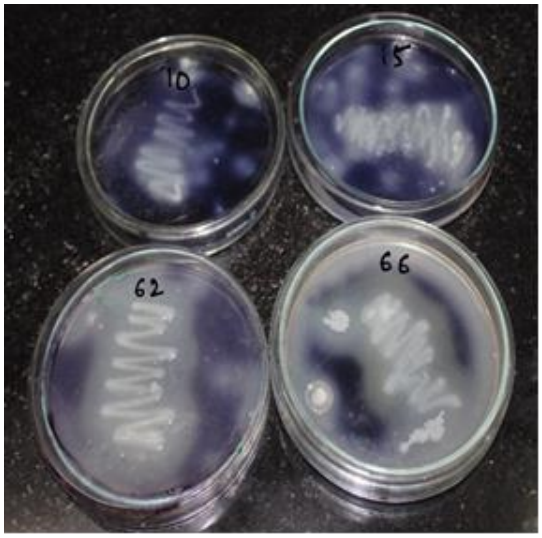


**Fig 3:** Comparison between isolates showing positive and negative response

**Fig 4:** Response to Indole production test

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